

| REPORT DOCUMENTATION PAGE | | | | Form Approved OMB No. 0704-0188 | |
|---|--------------------|---|-----------------------------------|---|--|
| Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS. | | | | | |
| 1. REPORT DATE (DD-MM-YYYY) 10 January 2007 | | 2. REPORT TYPE Final Performance Report | | 3. DATES COVERED (From - To) 1 Nov 2004 - 31 Oct 2006 | |
| 4. TITLE AND SUBTITLE Engineered Muscle Actuators: Cells and Tissues | | | | 5a. CONTRACT NUMBER FA9550-05-1-0015 | |
| | | | | 5b. GRANT NUMBER FA9550-05-1-0015 | |
| | | | | 5c. PROGRAM ELEMENT NUMBER | |
| 6. AUTHOR(S) Robert G. Dennis, Ph.D., Associate Professor of Biomedical Engineering | | | | 5d. PROJECT NUMBER | |
| | | | | 5e. TASK NUMBER | |
| | | | | 5f. WORK UNIT NUMBER | |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of North Carolina Office of Sponsored research At Chapel Hill UNC Chapel Hill Admin. Office Building, CB#1350 104 Airport Dr., Suite 2200 Chapel Hill, NC 27599-1350 | | | | 8. PERFORMING ORGANIZATION REPORT NUMBER | |
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Air Force Office of Sci. Res. 4015 Wilson Blvd, Arlington VA DARPA <i>Dr. Hugh DeLong/NL</i> | | | | 10. SPONSOR/MONITOR'S ACRONYM(S) AFOSR DARPA DSO | |
| | | | | 11. SPONSOR/MONITOR'S REPORT NUMBER(S) | |
| 12. DISTRIBUTION / AVAILABILITY STATEMENT Approve for Public Release: Distribution Unlimited | | | | | |
| 13. SUPPLEMENTARY NOTES | | | | | |
| 14. ABSTRACT We report the completion of Phase II of a technological development program for the production of living muscle mechanical actuators for robotic and prosthetic applications. Our primary objectives were to engineer living skeletal muscle actuators in culture using integrated bioreactors to guide tissue development and to maintain tissue contractility, to achieve 50% of adult phenotype muscular contractility, and then to install the engineered muscles into a centimeter-scale hybrid swimming robotic platform. Outcomes by milestone: (1) Develop integrated tissue culture bioreactor systems: completed all but bulk perfusion (2) Develop appropriate tissue interfaces in culture: full success, muscle-tendon & nerve (3) Achieve 50% of adult muscle functional capacity: excellent progress but not 50% (4) Swimming robotic platform with muscle: 50% success due to inadequate muscle performance Overall, this project resulted in many planned and collateral technological advances, including sub-cm scale cardiac muscle powered swimming actuators, functional tendon and nerve tissue interfaces, integrated rapid manufactured tissue bioreactors & improved contractility. | | | | | |
| 15. SUBJECT TERMS | | | | | |
| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON Robert G. Dennis, Ph.D. |
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | 19b. TELEPHONE NUMBER (include area code) 919-730-0221 (RG Dennis) |

DARPA Bio Molecular Motors Program, Phase II
Engineered Muscle Actuators: Cells and Tissues

Award number FA9550-05-1-0015

FINAL PERFORMANCE REPORT

Period: 1 November 2004 – 31 October 2006

Key Personnel and Institutions:

PI, Lead Institution: Robert Dennis, Ph.D. University of North Carolina at Chapel Hill
Tissue Engineering Systems Laboratory (TESLa)

Co-PI Subcontract: Hugh Herr, Ph.D. MIT Media Laboratory

Co-I Subcontract: Kevin Kit Parker, Ph.D. Harvard Div. of Eng. & Applied Sciences

Co-I Subcontract: Functional Tissue Engineering Lab at the University of Michigan:
Lisa Larkin, Ph.D., Institute of Gerontology, Director of the FTEL
Ellen Arruda, Ph.D. Dept. of Mechanical Engineering, U-Michigan

Co-I Subcontract: Keith Baar, Ph.D. University of Dundee (Scotland)

Table of Contents:

| Section | |
|---------|--|
| 1 | Cover Sheet, form SF 298 |
| 2 | Project Title, PI, P.O.C., and Key Personnel (this page) |
| 3 | Objectives |
| 4 | Status of Effort |
| 5 | Accomplishments/New Findings |
| 6 | Personnel Supported |
| 7 | Publications |
| 8 | Interactions/Transitions |
| 9 | New Discoveries, Inventions, or Patent Disclosures |
| 10 | Honors & Awards |
| 11 | Technical Images and Recent Data |

P.O.C.

Robert G. Dennis, Ph.D.
Associate Professor of Biomedical Engineering
The University of North Carolina at Chapel Hill
CB# 7575, 435 MacNider Hall
Chapel Hill, NC 27599-7575
Office Phone: 919-843-6076
Cell Phone: 919-730-0221
e-mail: bob@unc.edu
Fax: 919-966-2963

3. Objectives:

The objectives of this research remain unchanged from our original approved proposal. These are copied below for reference. Each task is delegated appropriately between the lead institution and the subcontracting groups.

Phase II Primary Objectives:

- (1) Develop an *in vitro* engineered muscle construct that exhibits 50% of adult phenotype functional capacity. Quantitatively this constitutes a specific force generation capacity of 100 kPa, and a power density of 25 W/kg of contractile tissue mass.
- (2) Using the engineered muscle in a next generation hybrid swimming robotic platform, quantitatively demonstrate the robot in terms of locomotory performance, controllability, metabolic economy and machine robustness.

Key Technical Milestones and Deliverables:

- (A) Second generation integrated tissue culture perfusion bioreactors
- (B) Second generation cm-scale hybrid swimming robotic platform & control methodologies
- (C) Guidance of engineered skeletal muscle tissue phenotype and function. Target: 50% of adult phenotype contractility

Major Milestones & Sub-Tasks

- (A) Second generation integrated tissue culture perfusion bioreactors
 1. Employ rapid manufacturing techniques for bioreactors
 1. accelerate system development
 2. increase number of parallel experiments to determine optimal protocols
 3. fast-track for future commercial or military system development
 2. Combine bulk perfusion, electrical, and mechanical stimulation in one unit
 1. miniaturized micro-power electrical stimulator and servo mechanism
 2. mechanism to apply hormonal stimulation transient pulses
 3. Advanced micro bulk perfusion system (media, blood substitutes)
 1. closed perfusion system and low fluid shear during pumping
 2. monitor key metabolic substrates during culture
 3. semi-permeable membrane for gas exchange and O₂ delivery
 4. Real-time quantitative assessment of tissue contractility
 1. actuator length, shortening velocity, acceleration, force generation
 2. data logging and real-time analysis, embedded micro-power control
 5. Control of electrical stimuli and environmental boundary conditions to guide tissue phenotype development
 1. modulation of electrical stimulus parameters based on feedback of muscle stress and strain
 2. application of compliant, viscoelastic muscle boundary conditions
- (B) Second generation hybrid swimming robotic platform
 1. Combined bulk perfusion and electrical stimulation in cm-scale swimming robot
 2. Advanced control interface and methodologies for the modulation of muscle electrical stimulation to achieve desired muscle contractile behaviors

3. Incorporation of tissue maintenance algorithms & system health monitoring
4. System quantitative performance measurement
5. Incorporate engineered skeletal muscle (50% adult phenotype target)
6. Quantitative comparison between cell-cultured and organ-cultured muscle actuators

(C) Guidance of skeletal muscle tissue phenotype and function

1. Maximization of tissue formation efficiency: 2-D to 3-D
2. Localization of critical cellular components for tissue formation
3. Guidance & control of tissue fiber architecture in final 3-D structure
4. Assessment of fiber and tissue phenotype
 1. Propagation of action potential waves
 2. Calcium handling mechanisms during development
 3. Molecular and structural markers of tissue development
5. Electrical and mechanical stimulation – control of development & phenotype
 1. Determination of underlying cellular mechanisms in response to electrical stimulation to allow rational design of electrical stimulation protocols, and energetic optimization of the stimulation protocols
 2. modulation of electrical stimulus parameters based on feedback of muscle stress and strain
 3. application of compliant, viscoelastic muscle boundary conditions modeled after in vivo muscle operating conditions
6. Determine optimal protocols to emulate fast and slow motor nerves
7. Identify molecular targets that respond to electrical stimulation protocols
8. Development of critical functional tissue interfaces in culture
 1. Simulate or engineer, as necessary, nerve and tendon interfaces
 2. Quantify contractility and controllability of engineered constructs in culture

4. Status of Effort: Final Outcomes (summary)

Our research effort remained on target both in terms of milestone accomplishments according to schedule, as well as remaining within the planned budget, until the late stages of the effort. Overall we achieved many important technical milestones toward the development of fully-functional living engineered muscle actuators. Our primary failure during this research effort was our inability to achieve 50% of adult skeletal muscle contractility, which prevented the installation of the engineered muscle actuators into the hybrid robotic platform that was developed in parallel at MIT. The 50% contractility target was considered highly aggressive but achievable during our initial project negotiations, and though we failed to achieve this very difficult objective within the project period, the overall final milestone success rate was greater than we had initially anticipated.

5. Accomplishments/New Findings:

The primary findings of this research relate to our understanding of the generation of functional musculoskeletal and cardiovascular tissues. Our technical approach focused on musculoskeletal tissues at the system level, the development of key support technologies for the culture, control, guided development, and non-destructive testing

(NDT) of tissues and tissue systems in culture, the establishment of NDT biomarkers for tissue development, and the development of key tissue interfaces to allow the assembly of tissue elements into functional tissue systems. This is highly relevant to both the development of bio-hybrid (living-synthetic) systems, as well as for the pressing need for effective technology development in the area of regenerative medicine for Combat Casualty Care. The results of this technology are already beginning to have a positive impact on civilian technology for ex-vivo tissue engineering in the areas of musculoskeletal and cardiovascular functional tissue engineering and regenerative medicine, and the control of prosthetic devices (see sections 8.b and 8.c below). Images with our most recent findings and technology demonstrations are included in Section 11 at the end of this document.

6. Personnel Supported:

Lead Institution: *UNC – Chapel Hill, Tissue Engineering Systems Laboratory (TESLa)*
Robert Dennis, Ph.D. PI, Faculty and Director of the TESLa
Lauren Woods, Graduate Student Research Assistant, UNC BME

Subcontract: MIT *MIT Media Laboratory*
Hugh Herr, Ph.D., Faculty and Co-PI
Waleed Farahat, Graduate Student Research Assistant
Danielle Chou, Graduate Student Research Assistant
Ken Pasch, Technical Staff

Subcontract: Harvard *Harvard Division of Engineering & Applied Sciences*
Kevin Kit Parker, Ph.D., Faculty
Sean Sheehy, Professional Staff
Adam Feinberg, Postdoctoral Fellow

Subcontract: U-Mich *Functional Tissue Engineering Lab at the University of Michigan*
Lisa Larkin, Ph.D., Faculty, Inst. Gerontology, Director of the FTEL
Ellen Arruda, Ph.D. Faculty, Dept. of Mechanical Eng., U-Michigan
Fatima Syed, Graduate Student Research Assistant,
Jonathan Andrick, Student Laboratory Assistant
Dustin Cummings, Student Laboratory Assistant
Jeff Kennedy, Student Laboratory Assistant
Amy Taylor, Student Laboratory Assistant

Subcontract: Dundee Keith Baar, Ph.D. University of Dundee (Scotland)

7. Publications:

Manuscripts Submitted

- i. Kosnik, P.E., Dennis R.G. Contractility and myosin heavy chain content of skeletal muscle engineered from adult and aged rats. In Review: Tissue Engineering.
- ii. Dennis, R.G., Dow, D. E. Excitability of skeletal muscle during development, denervation, and tissue culture. Submitted to Tissue Engineering on 10-14-2006.

- iii. Birla RK, Borschel, GH, Dennis, RG, Brown, DL. In vivo vascularization of tissue engineered heart muscle. (Submitted to Tissue Engineering).

Manuscripts Accepted for Publication

- i. Larkin, L.M., Calve, S. Kostrominova, T.Y. and Arruda, E.M. Structural and Functional Evaluation of Tendon-Skeletal Muscle Constructs Engineered in Vitro. Accepted for publication in Tissue Engineering May 2006.
- ii. Larkin, L.M. Van der Muelen, J H. Dennis, R.G. and Kennedy, J.B. Functional Evaluation of Nerve-Skeletal Muscle Constructs Engineered in Vitro. Accepted for publication in In vitro Cell. Dev. Biol. December 2005.
- iii. Arruda E.M., K. Mundy, S. Calve,,and K. Baar. Denervation does not change the ratio of collagen I and collagen III mRNA in the extracellular matrix of muscle. Am J Physiol Endocrinol Metab. Oct 3; [Epub ahead of print]

Manuscripts Published

- i. Lockhart, N.C., K. Baar, R.S. Mazzeo, and S.V. Brooks. Activation of Akt as a potential mediator of adaptations that reduce muscle injury. Med. Sci. Sports Excer. 38:1058-1064. 2006.
- ii. Dennis B., Herr H. "Engineered Muscle Actuators: Cells & Tissues," *Biomimetics Mimicking and Inspired by Biology*, Bar-Cohen, Y., CRC Press; 2005.
- iii. Farahat W., Herr H. An Apparatus for Generalized Characterization and Control of Muscle. IEEE Transactions on Neural Systems & Rehabilitation Engineering. 2005; 13(4): 473-481.
- iv. Farahat W., Herr H. A Method for Identification of Hammerstein Models of Electrically Stimulated Muscle. 27th Annual International Conference of the IEEE Engineering in Medicine and Biology Society; Shanghai, China; September 1-4, 2005.
- v. Farahat W., Herr H. Workloop Energetics of Antagonist Muscle Pairs. 28th International Conference of the IEEE Engineering in Medicine and Biology Society, New York, 2006.
- vi. Arruda, E.M., Calve, S., Dennis, R.G., Mundy, K., Baar, K. Regional variation of tibialis anterior tendon mechanics is lost following denervation. J. Appl. Physiol. 101:4 1113-17, May, 2006. PMID: 16728516.
- vii. Borschel, G.H., Huang, Y.C., Calve, S.C., Arruda, E.M., Lynch, J.B., Dow, D.E., Kuzon, W.M., Dennis, R.G., and Brown, D.L., Tissue Engineering of Recellularized Microvascular Grafts, Tissue Engineering, 11:5/6 778-786, 2005.
- viii. Baar K. New dimensions in tissue engineering: possible models for human

physiology. *Exp Physiol*. 90:799-806. 2005.

- ix. Birla RK, Borschel GH, Dennis RG. In vivo conditioning of tissue-engineered heart muscle improves contractile performance. *Artificial Organs*, Vol. 29, No. 11, 866-875, Nov. 2005.
- x. Baar K, Birla R, Boluyt MO, Borschel GH, Arruda EM, Dennis RG. Self-organization of rat cardiac cells into contractile 3-D cardiac tissue. *FASEB J*. 19(2):275-7, 2005, doi: 10.1096/fj.04-2034fje, Full text available on-line at: <http://www.fasebj.org/cgi/doi/10.1096/fj.04-2034fje>
- xi. Huang, Y., Dennis, R.G., Baar, K. Cultured slow vs. fast skeletal muscle cells differ in physiology and responsiveness to stimulation. *Am J. Physiol – Cell Physiol*, 291(1): C11-C17, July, 2006.
- xii. Larkin, L.M., Van der Meulen, J. H., Dennis, R. G., Kennedy, J. B. Functional evaluation of nerve-skeletal muscle constructs engineered in vitro. *In Vitro Cellular and Developmental Biology – Animal*, 42(3-4): 75-82, 2006.
- xiii. Borschel GH, Dow DE, Dennis RG, Brown DL. Tissue engineered axially-vascularized contractile skeletal muscle. *Plastic and Reconstructive Surgery*, 117(7): 2235-42 June 2006.
- xiv. Huang, Y-C, Robert G. Dennis, Lisa Larkin, and Keith Baar. Rapid formation of functional muscle in vitro using fibrin gels. *J Appl Physiol* 98: 706–713, 2005.
- xv. Herr, H., and Dennis, R.G. A swimming robot actuated by living muscle tissue. *J Neural Eng & Rehab*, 1(6) Sept 2004, doi: 10.1186/1743-0003-1-6.
- xvi. Birla, R.K., Borschel, G.H., Dennis, R.G., Brown, D.L., Myocardial Engineering In Vivo: Formation and Characterization of Contractile, Vascularized 3-Dimensional Cardiac Tissue. *Tissue Engineering*, 11: 5/6, 803-813, 2005.

8. Interactions/Transitions:

a. Participation/presentations at meetings, conferences, seminars, etc.

- i. Dennis, R.G. Muscle engineered from satellite cells of aged rats: contractile function, excitability, and structure. World Congress of Biomechanics (WCB) 5th world congress of biomechanics, Munich, Abstract # 6751, Lecture Track 9: Tissue Engineering, July 19- August 4, 2006.
- ii. Dennis, R.G. and Kosnik, P.E. Time course of in vitro development of engineered skeletal muscle: contractility and myosin heavy chain content. World Congress of Biomechanics (WCB) 5th world congress of biomechanics, Munich, Abstract # 6727, Lecture Track 9: Tissue Engineering, July 19- August 4, 2006.
- iii. Dennis RG, Arruda EM, Birla RK, Baar K, Larkin LM, Kosnik, PE. Self-

organization and contractile function of 3-dimensional striated muscle engineered in culture. World Congress of Biomechanics (WCB) 5th world congress of biomechanics, Munich, Abstract # 6725, Lecture Track 2: Musculoskeletal Mechanics-Joint ISB Track, July 19- August 4, 2006.

- iv. Dennis RG. Engineered, self-organizing living muscle actuators. MRS: Materials Research Society Spring Meeting, Paper# AA3.6, San Francisco, CA, April 17-21, 2006.
- v. Kostrominova, T.Y. Calve, S. Larkin, L.M. Arruda, E.M. Myotendinous junction protein expression in engineered muscle-tendon constructs. Experimental Biology 2006
- vi. Calve, S Kostrominova, T.Y. Arruda, E.M. Larkin, L.M. Functional Evaluation of Engineered Three-Dimensional Muscle-Tendon Constructs. Experimental Biology 2006
- vii. Kostrominova, T.Y , Calve, S. Arruda, E.M. Larkin, L.M. Myotendinous Junction Protein Expression in Engineered Muscle-Tendon Constructs. Biomedical Engineering Society Meeting, Tissue Engineering & Biomaterials, Oct 2006
- viii. Calve, S. Kostrominova, T.Y Arruda, E.M. Larkin, L.M. Functional Evaluation of Engineered Three-Dimensional Muscle-Tendon Constructs. Biomedical Engineering Society Meeting, Tissue Engineering & Biomaterials, Oct 2006
- ix. Calve, S., Arruda, E.M., Mundy, K., Dennis, R. and Baar, K., "The Effect of Denervation and Aging on the Heterogeneous Material Properties of the Tibialis Anterior Tendon," XXth Congress of the International Society of Biomechanics, July 31 - August 5, 2005, Cleveland, OH.
- x. Calve, S.C., Syed, F.N., Dennis, R.G., Grosh, K., Garikipati, K., and Arruda, E.M., "Mechanical Characterization of Growth in Fibrin-Based Tendon Constructs," 2005 Summer Bioengineering Conference, June 22-26, 2005, Vail, CO.
- xi. Calve, S.C., Baar, K., Mundy, K., and Arruda, E.M., "The Effect of Denervation on the Heterogeneous Material Properties of the Tibialis Anterior Tendon," 2005 Summer Bioengineering Conference, June 22-26, 2005, Vail, CO.
- xii. Calve, S., Baar, K., Narayanan, H., Garikipati, K., Grosh, K., Dennis, R.G. and Arruda, E.M., "Development of Constitutive Models to Describe Growth in Soft Tissues: Experimental Basis," McMat 2005, June 1-3, 2005, Baton Rouge, LA.
- xiii. Farahat W., Herr H. A Method for Identification of Hammerstein Models of Electrically Stimulated Muscle. 27th Annual International Conference of the IEEE Engineering in Medicine and Biology Society; Shanghai, China; September 1-4, 2005.
- xiv. Lisa M. Larkin, Robert G. Dennis, Jeff Kennedy, and Jack D. Van der muellin. Functional Evaluation of Engineered Three-Dimensional Nerve-Muscle Constructs. Experimental Biology, Spring 2005

b. Consultative and advisory functions to other laboratories and agencies.

- i. The PI (Robert Dennis) has consulted for Tissue Genesis, Inc (Honolulu, HI) in their development of tissue culture bioreactors for ligaments and large-diameter blood vessels. Though not directly related to our DARPA/AFOSR research, the

technology and technical know-how resulting from our research under the AFOSR project was pivotal in the successful development of the TGI technology. The technical contact at TGI is Dr. Paul Kosnik.

- ii. MIT's stationary bioreactor for muscle control and identification was used by Dr. Ratna from the Center for Bio/Molecular Science and Engineering Code 6930, Naval Research Laboratory 4555, Overlook Avenue SW Washington DC.

c. Transitions.

- i. Tissue Genesis, Incorporated (Honolulu, HI) has licensed the technology for our fibroid invention disclosure and is using it in an effort to engineer ligament from bone marrow stem cells. Tom Cannon is the TGI administrative contact.
- ii. Development of bioreactor subsystems for ligament and large diameter blood vessel perfusion bioreactors at Tissue Genesis, Inc. (see 8.b.i, above)

9. New discoveries, inventions, or patent disclosures.

- i. Farahat W., Herr H., inventors; An Apparatus for Generalized Characterization and Control of Muscle. Pending 2004.
- ii. Woods, L., MacDonald, J., Dennis, R.G., Seagle, C. Scalable NMR Compatible Bioreactor (UNC, 2005).

10. Honors/Awards:

- i. H. Herr (MIT): Best Invention of the Year, Time Magazine 2004
- ii. H. Herr (MIT): Popular Mechanics Breakthrough Leadership Award 2005
- iii. H. Herr (MIT): NEC Career Development Professorship 2005
- iii. R.G. Dennis (UNC): Visiting Professor, Faculty of Dentistry, Tissue Engineering Laboratories, Alexandria University, Alexandria, Egypt. 2006
- iv. Pfizer Prize from the Physiological Society (London). Award Lecture entitled "New dimensions in tissue engineering: possible models for human physiology."

11. Technical Images and Recent Data

SUMMARY OF RESULTS:

The following information is a summary of progress during the final 12 month period of the project (1 November 2005 through 31 October 2005). In some cases these results are significant improvements over earlier results in this study, from Phase I, the Bridge Period, or the first half of Phase II.

MAJOR TASK A: Second generation integrated tissue culture perfusion bioreactors

The UNC group led the development of the final version of the integrated cell culture bioreactor. The system was rapid manufactured using primarily the fusion deposition modeling process with polycarbonate material. This allowed us to simultaneously test up to 77 engineered tissue specimens by the close of the project, which is an order of magnitude greater than has been reported by any other group in the literature.

We combined electrical and mechanical tissue stimulation into each bioreactor system, and each could maintain up to 11 individual engineered muscle specimens in culture.

We achieved real-time monitoring of muscle specimen electrical and mechanical stimulation (all of the proposed parameters except real-time force generation), these included actuator length, shortening velocity, specimen strain, and acceleration, with data logging and real-time analysis and embedded micro-power control.

The MIT group developed technology to control electrical stimuli and environmental boundary conditions based on feedback of muscle stress and strain, and the ability to apply compliant, viscoelastic muscle boundary conditions. This was done on whole muscle explants to maintain adult phenotype for the longest period possible.

Failures: the integration of perfusion was not successful in the cell culture bioreactor architecture. Improved designs will allow perfusion to be incorporated, but not within the Phase II period.

MAJOR TASK B: Second generation hybrid swimming robotic platform

This task was carried out primarily by the MIT group with assistance from the UNC group.

The primary accomplishment in this major task was the development of the advanced control interface and methods for the coordinated control of the muscle electrical stimulation and mechanical boundary conditions.

There was further development of the advanced cm-scale hybrid swimming robotic platform.

Failures: Full achievement of the stated goals of this major task were hindered by the failure of our overall objective to achieve sufficient phenotype (50% of adult contractility) to power the robotic platforms as designed. This caused a failure of convergence of these parallel research efforts toward the final technological goal. Good progress was made, but this key objective turned out to be beyond our capability in the period of performance.

MAJOR TASK C: Guidance of skeletal muscle tissue phenotype and function

This major task was the most integrated in terms of cross-over between all of the research groups (UNC, U-Michigan, Harvard, MIT, and Dundee).

The Harvard group was able to develop a method for making functional muscle actuators on patterned substrates. They did increase the alignment efficiency, but not for 2-D to 3-D scaffold-free tissue self-organization as originally intended. The patterned substrate was actually incorporated into the final actuator structure.

The UNC and Dundee groups worked together on the coordination of electrical, chemical and mechanical stimulation. These were used successfully in a coordinated effort to enhance the muscle tissue phenotype, but the desired final value of 50% of adult phenotype was not achieved. The various combinations we employed tended to have a summation of effects when multiple stimuli were coordinated, but generally the enhancements were at best on the order of 4 to 5 fold improvements over the baseline culture conditions with no stimuli.

The UNC and U-Michigan groups had excellent success in the development of both of the proposed critical tissue interfaces: muscle-tendon and nerve-muscle. In both cases the tissue interfaces had molecular markers specific to the interface, and in both cases the tissue development in culture was enhanced. This was the highest-impact biological result from this research.

Failures: We failed to achieve our overall objective of 50% of adult skeletal muscle contractility in the engineered muscle tissues. This was a highly aggressive goal, and we made good progress, but were unable to achieve it within the project period.

Global impact of this work:

The scientific and technological work in this project had two major impacts on research in the field of musculoskeletal tissue engineering:

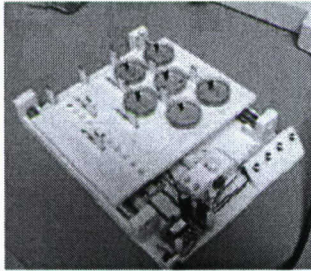
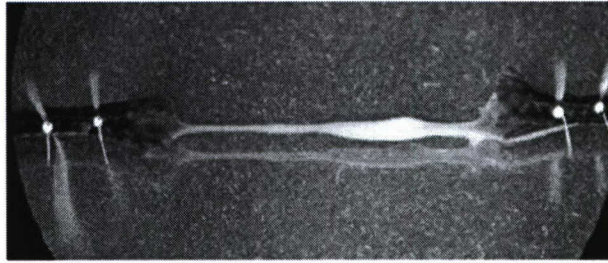
- 1- Our results have demonstrated the value of the development and use of functional tissue interfaces in current research in musculoskeletal tissue engineering. This was one of our stated goals, and at the outset of our work there was considerable resistance to this strategy from within the academic research community. Subsequently, the U-Michigan group has recently won NIH RO1 funding to continue this line of research.
- 2- Our technological approach and results have clearly had an impact on funding emphasis within NIH, as there is a markedly increased interest in enabling technologies for tissue engineering research. The direct and fundamental impact of our work developing integrated, modular bioreactor systems in the DARPA-BMM program is readily apparent in the text of the RFA for NIH PAR-06-504: Enabling Technologies for Tissue Engineering and Regenerative Medicine (R01)

Engineered Functional Muscle Tissue and Interfaces (UNC, U-Michigan, Dundee)

During the course of the project, this group accomplished several very important technical advances that will have a major impact on current and future musculoskeletal tissue engineering. These include:

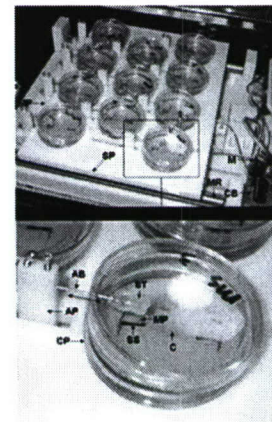
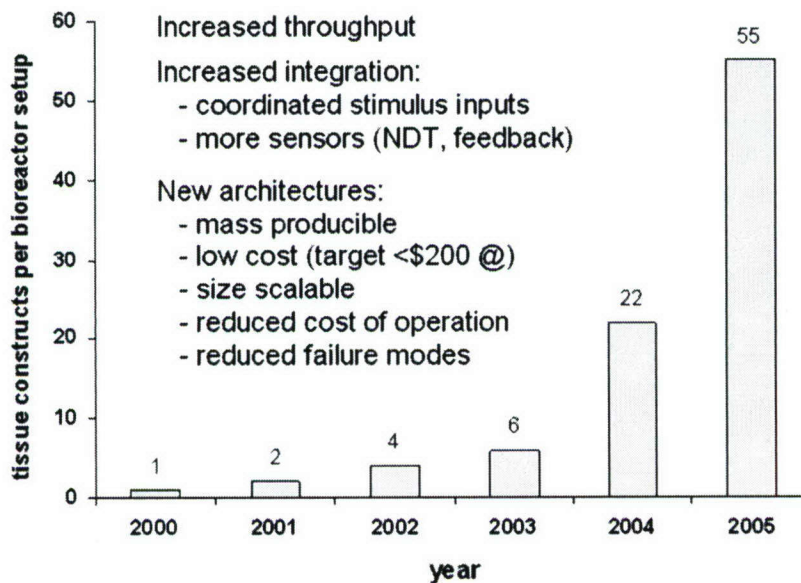
- (A) The first demonstration of a functional nerve-muscle tissue interface engineered in vitro in which an action potential generated in the axons of cultured nerves resulted in synaptic transduction of the action potential from the nerve to the muscle with subsequent muscular contraction
- (B) The first demonstration of a self-organizing tendon construct engineered in vitro from primary mammalian cells
- (C) The first demonstration of developmental improvements in engineered tendon constitutive properties (stress-strain behavior) for engineered tendon maintained in culture (manuscript in preparation)
- (D) The first use of rapid-manufactured bioreactors to apply controlled electro-mechanical stimulation to engineered tissues in culture, resulting in enhanced contractility (manuscript in review)
- (E) The first demonstration of a functional muscle-tendon interface engineered in culture (manuscript accepted).

As a group our emphasis has been on the development of robustly repeatable technologies to create and functionally evaluate 3-dimensional musculoskeletal and cardiovascular tissue constructs and tissue-tissue interfaces.



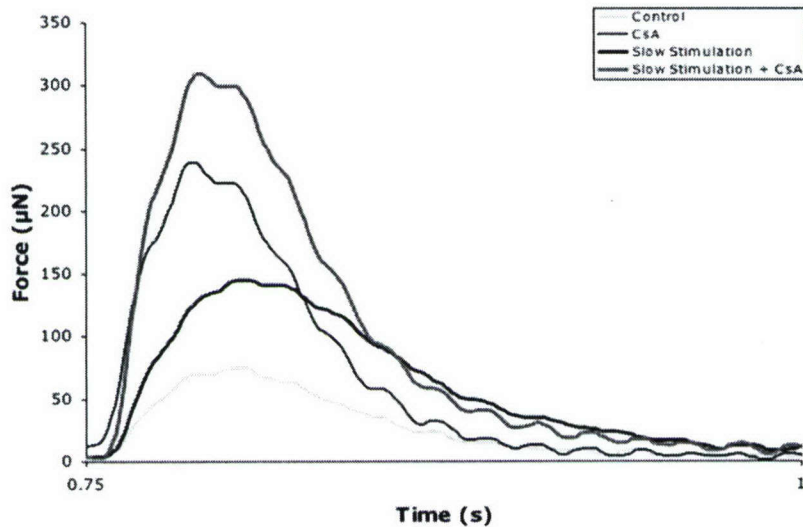
Upper Left: first report of functional nerve-muscle interface
 Above: first report of functional self-organizing cardiac muscle
 Left: bioreactor systems for engineered muscle
 Not shown: Functional muscle-tendon tissue interface
 Self-assembling cardiac tissue-based pump
 Next-generation integrated bioreactor systems
 Functional assessment of muscle tissues in culture

Tissue Bioreactor Throughput



Approximates Moore's Law for semiconductor density per unit area
 Continued progress necessitates a radical new architecture for bioreactors

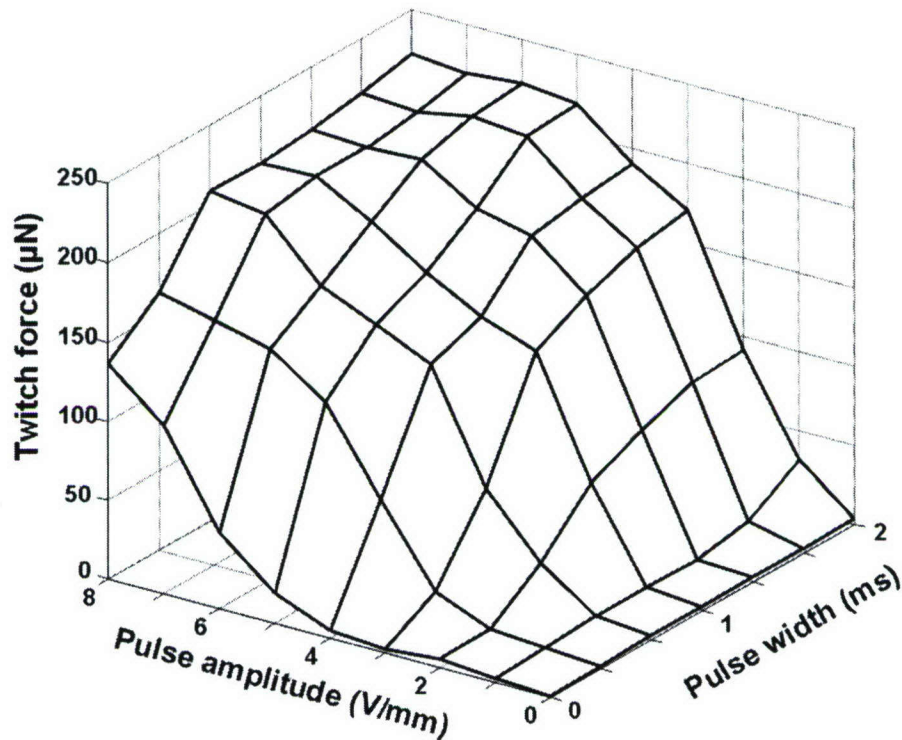
Combined interventions: Elect Stim + Chem Stim Yields 4-Fold Force Improvement



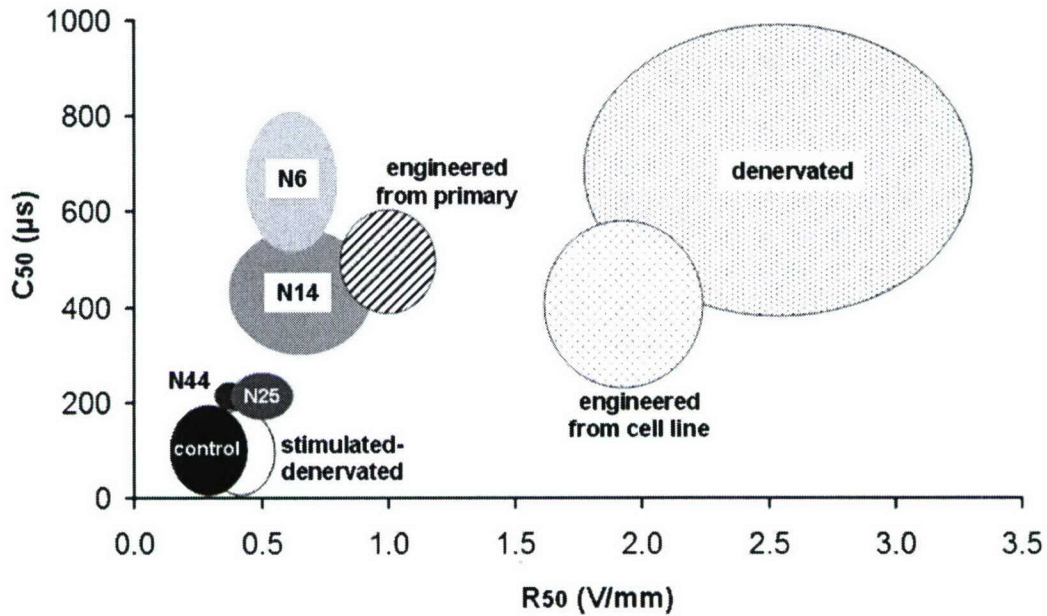
Keith Baar (U-Dundee)

The effects of cyclosporine A (blocking calcineurin) + slow stimulation.

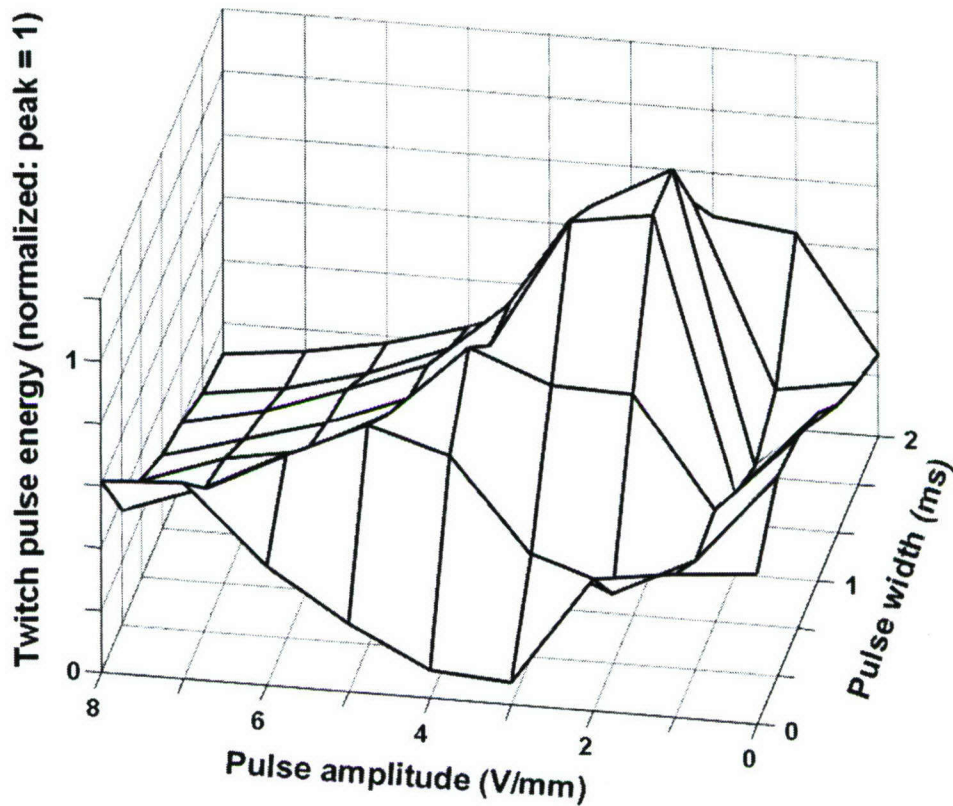
- Electrical stimulation alone stimulation doubles force production
- CsA triples force production and speeds contraction by 28%
- Both interventions increased force production 4-fold and sped contraction 30%



Muscle tissue excitability “mesh plot”, showing the effect of the two independent variables (pulse amplitude and pulse duration) on twitch force. The data shown are for a single myooid engineered from myogenic cells from the soleus muscle of an adult rat (experimental group #2). Although this surface gives an accurate and more complete description of the bulk excitability of the tissue specimen, it is desirable describe the tissue excitability with the fewest possible number of metrics. This is the reason for developing the values R_{50} and C_{50} , based upon classical physiological measurements of cell excitability, have been reported. (data file: myooid-A138).



Reohbase (R_{50}) and Chronaxie (C_{50}) values for 9 experimental groups plotted in “excitability space” as a bubble plot. This representation provides a quantitative summary of both the mean and standard deviation for each experimental group for R_{50} and C_{50} , as well as a qualitative sense for the data variability within each group and the relative differences in excitability between different experimental groups. Each group is represented as an ellipse, with the horizontal axis of each ellipse equal to the mean $R_{50} \pm$ one standard deviation, and the vertical axis for each ellipse equal to the mean $C_{50} \pm$ one standard deviation. Nx represents the excitability of neonatal rat muscle (x days post partum). Other ellipses are labeled according to the experimental group to which they correspond in Table 1, where “myooid” indicates an *in vitro* engineered skeletal muscle, either from primary rat cells, or from the C2C12 cell line



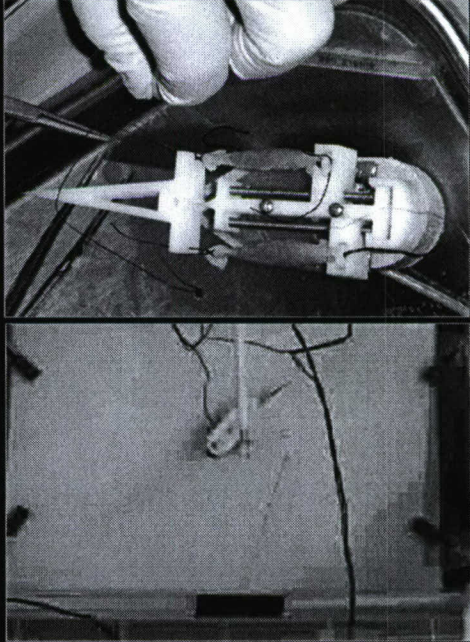
To determine the appropriate stimulation parameters for engineered muscle, it is necessary to determine the optimal stimulation in terms of energy output divided by stimulation energy input. Optimal stimulation is defined for maximal twitch force per unit stimulus pulse energy. These data are from the same myooid specimen shown in the previous figure. Note that the peak force output per unit stimulus energy input does not correspond with stimulus parameters that generate peak twitch force, which generally occurs at the highest pulse amplitude and width. Rather, for this engineered muscle specimen the optimal stimulation occurs at a pulse amplitude of ~ 1.5 to 2.0 V/mm and a pulse width of 0.8 to 1.0 ms. The mean excitability for myooids in this group (group #2) was: $R_{50} \sim 1$ V/mm and $C_{50} \sim 0.5$ ms. Thus, for this specimen the optimal stimulation occurs approximately at double the values for R_{50} and C_{50} . (data file: myooid-A138).

Next-Generation Hybrid Swimming Robotic Platform (MIT: Hugh Herr)

Muscle-Actuated Robotic Platform

Specifications

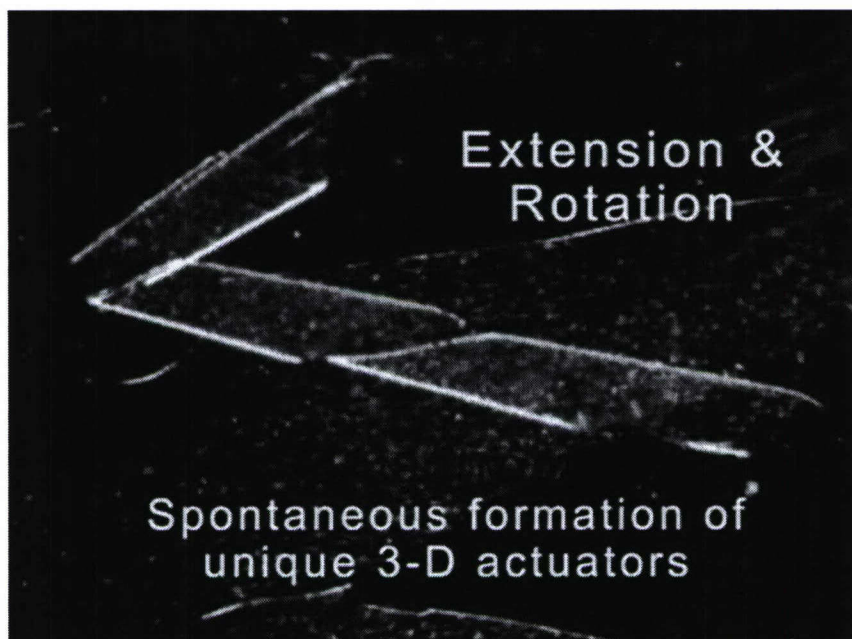
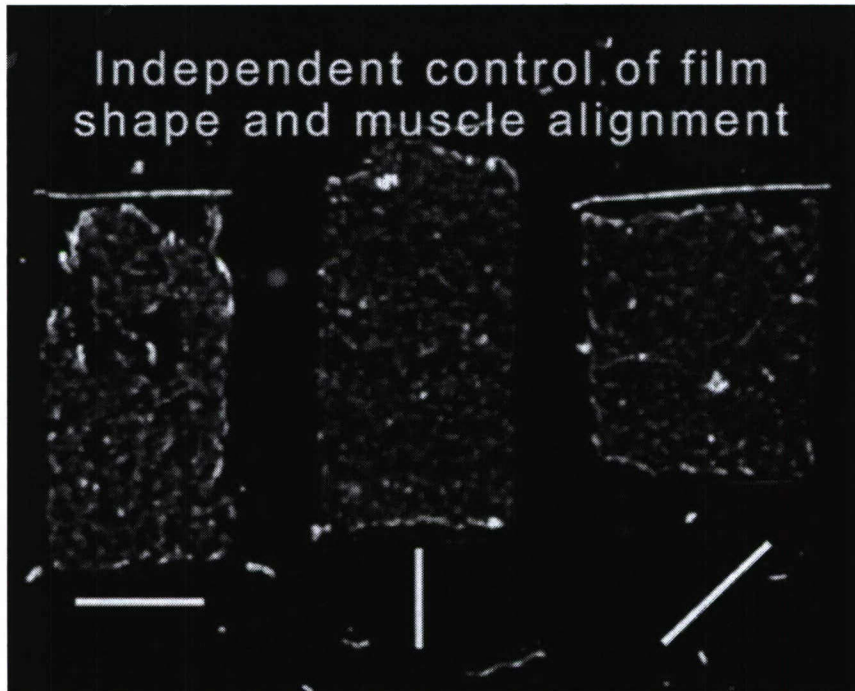
- **Single oscillating tail**
(For $V < 1.5$ lengths/sec only
posterior muscles active in Bass and Trout, *JEB*, 1995, Jayne & Lauder)
- **On-board power and computation**
- **Wire electrodes**
- **Sensing of tail position**
- **Adjustable muscle equilibrium position**

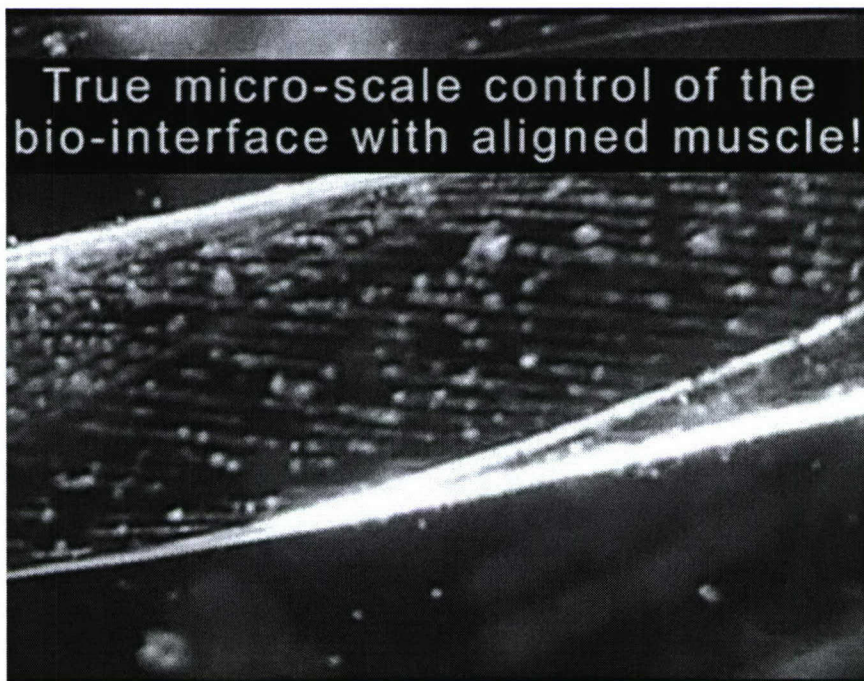
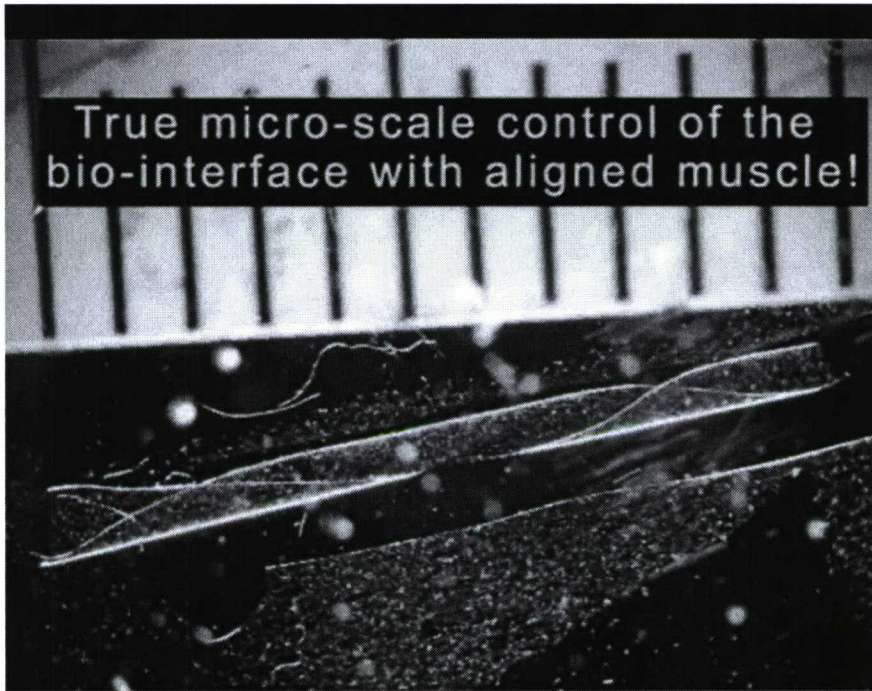


The MIT effort focused primarily on control interface issues and the development of a next-generation hybrid robotic platform to demonstrate the 50% adult phenotype muscle. The failure to achieve this level of contractility in the parallel experiments by the conclusion of our proposal period meant that the hybrid robot was evaluated using explanted whole muscles from frogs. It was understood that achieving 50% adult contractility in engineered muscle tissue was a “DARPA hard” problem with a high probability for failure within the project period. Nonetheless, our many key and enabling technological accomplishments during the project period have paved the way for success in this general area, probably within the next several years.

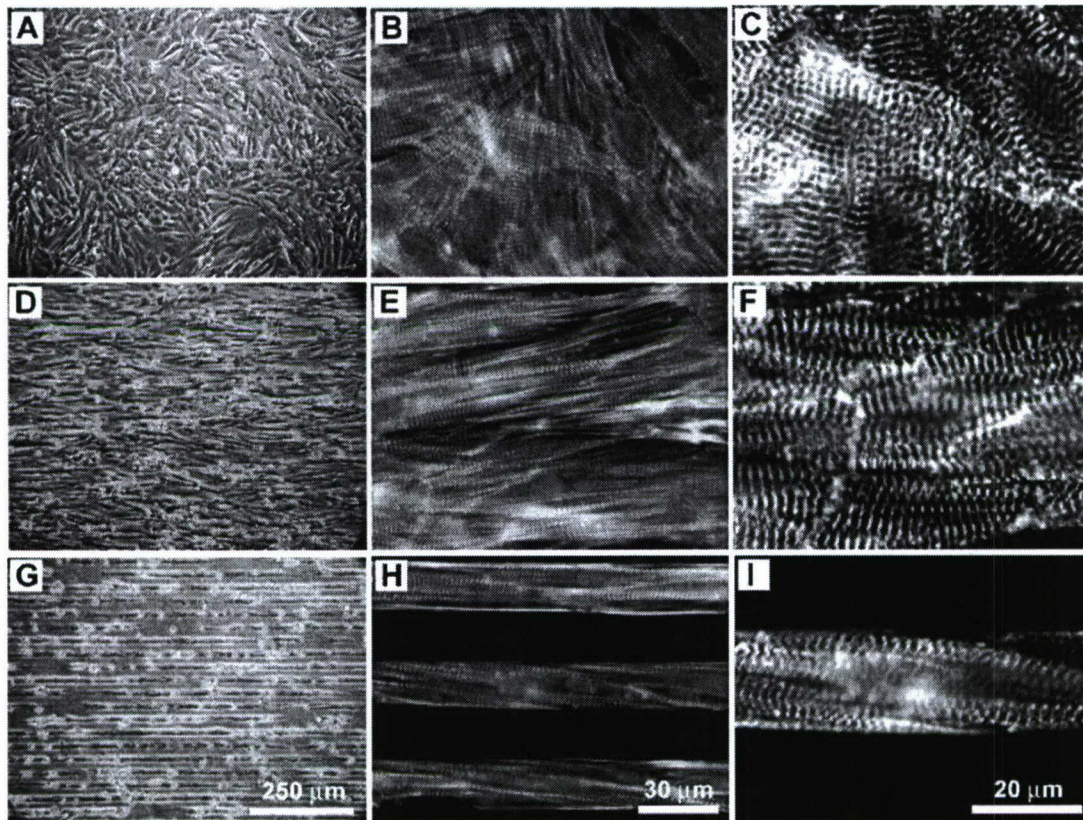
MUSCULAR THIN FILMS (Harvard: KK Parker)

Images from the movie in real time showing dynamic contractility
(movie file is available upon request: bob@unc.edu)

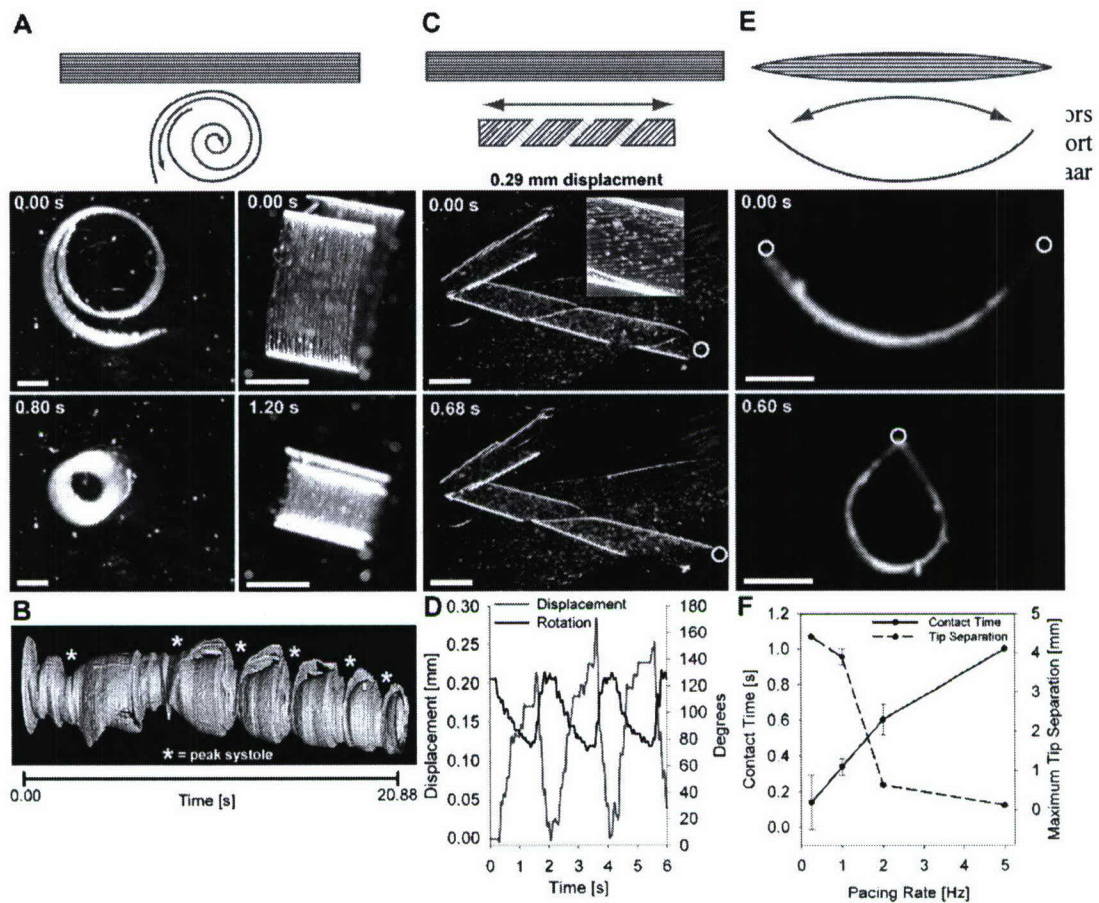




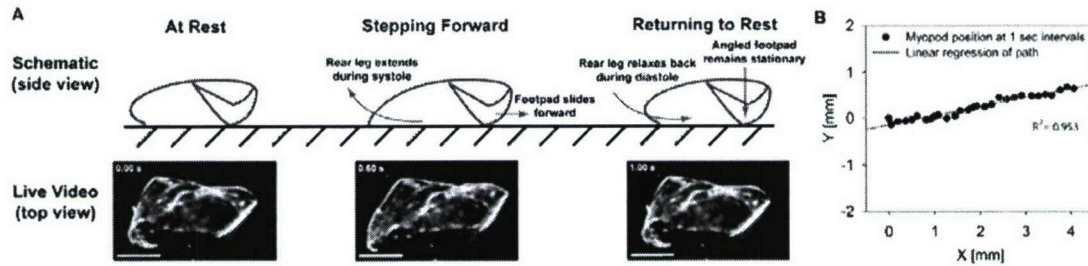
MTF (muscle thin film) data in preparation for publication:



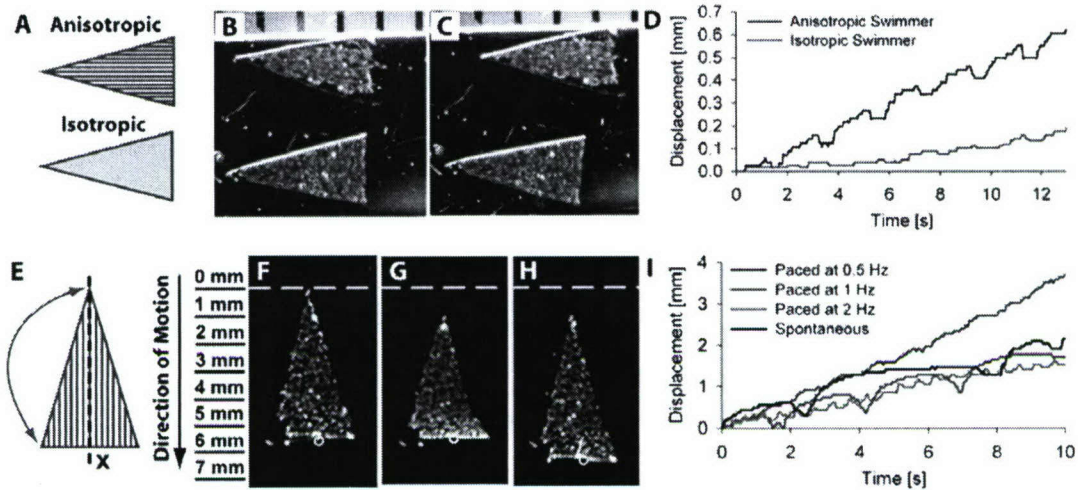
Uniform FN coatings produce isotropic 2D myocardium (A, B and C) with no long-range order. (C) Staining for sarcomeric α -actinin reveals no preferential alignment of sarcomeres along any axis. Micropatterns of alternating high and low density 20 μ m wide FN lines (D, E and F) produce continuous anisotropic 2D myocardium. (F) Staining for sarcomeric α -actinin reveals uni-axial alignment of sarcomeres along a single axis. Micropatterns of alternating 20 μ m wide lines of high density FN and Pluronics (G, H and I) produce arrays of anisotropic 1D myocardial strips. (I) Staining for sarcomeric α -actinin reveals uni-axial alignment of sarcomeres along a single axis. Images are 10x phase (A, D and G); 63x immunofluorescence (B, E and H) of nuclei (blue), F-actin (green) and sarcomeric α -actinin (red); and the signal from sarcomeric α -actinin alone (C, F and I) to indicate and emphasize the direction of sarcomere alignment.



These are soft robotic actuators created from MTFs. (A) The coiled strip is a rectangle with anisotropic myocardium (on the concave surface) that is aligned along the length and transitions between coiled and un-coiled states during spontaneous myocyte contraction. (B) The top-down view of the transition from uncoiled to coiled is rendered in 3D as function of time to illustrate the more rapid coiling rate (contraction) relative to the slower uncoiling rate (relaxation), peak systole is denoted by the yellow '*'. (C) The helical linear actuator is a rectangular strip with anisotropic myocytes (on the convex surface) aligned $\sim 5^\circ$ off-axis to the length. It spontaneously adopts a helical conformation, rotating and lengthening (extension) during myocyte contraction. (D) Tracking the tip of the helical linear actuator (yellow circles in video still images) shows cyclic extension and rotation when externally paced at 0.5 Hz. (E) The gripper is a thin rectangular strip with anisotropic myocytes aligned along the length (and on the concave surface) that brings the tips together upon myocyte contraction. (F) Tracking the relative distance between the tips (yellow circles in video still images) as a function of pacing rate demonstrates that we can control (i, dashed line) the maximum tip separation during diastole and (ii, solid line) the average time the gripper is closed over a 1 sec period (error bars represent standard deviation). For each construct type, a schematic is given of the shape prior to release from the cover slip with anisotropic myocyte alignment indicated by blue lines and isotropic myocytes indicated by uniform blue shading. Side profiles demonstrate the 3D conformation the films adopt upon release from the cover slip and the direction of film bending is indicated by the red arrows. The video still images show the construct in a relaxed state at time 0.00 s and in a contracted state 0.60 to 1.20 s later. Scale bars are 1 mm.



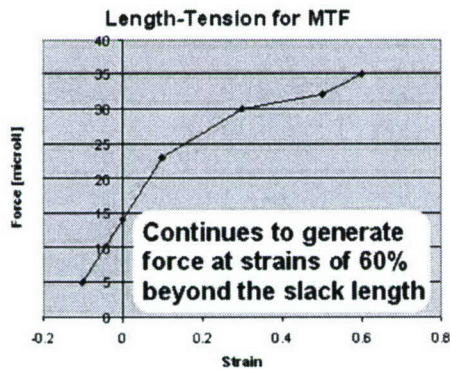
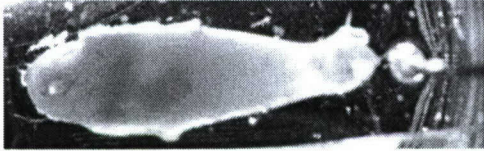
The myopod is formed from a triangle MTF with isotropic myocardium (on the convex surface) and is manually folded into a 3D shape. (A) The walking motion of the myopod, is illustrated in a schematic view from the side and live view from the top as it starts in diastole and then steps forward during systole. The foot pad (right side of the myopod) touches the substrate at an angle allowing the myopod to slip easily to the right and resist slip to the left. During systole the myocytes on the convex side of the MTF contract causing the rear 'leg' to extend and pushing the myopod to the right. As the rear 'leg' relaxes back during diastole the angled foot pad prevents the myopod from slipping back to the right resulting in net displacement to the right. Analysis of video frames shows that the myopod walks across the bottom of the Petri dish by extending its rear leg from a relaxed (0.00 s) to contracted (0.60 s) to relaxed (1.00 s) state at 1 Hz pacing rate. (B) Frame by frame video tracking of the front of the myopod at 1 second intervals shows consistent and directed locomotion. When paced at 1 Hz the average speed is ~8 mm/min. Scale bars are 1 mm.



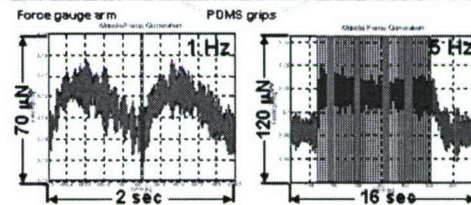
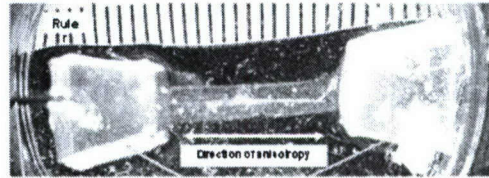
A triangular MTF swimmer demonstrates how tissue microstructure, film shape and pacing rate collectively contribute to motility. (A) We compared the motility of similarly shaped triangle swimmers with anisotropic and isotropic tissue structure paced at 0.5 Hz. When pacing commences (B), the anisotropic triangle swimmer surges ahead after 13 sec of pacing (C). (D) Tracking displacement of both swimmers frame-by-frame shows that the anisotropic swimmer is ~5 times faster than the isotropic swimmer over the 13 sec. (E) The MTF anisotropic triangle swimmer is realized by aligning myocytes parallel to the height of the triangle. The myocyte alignment is indicated by the blue lines and the direction of film bending is indicated by the red arrows, which denote the points that bend down, into the plane of the paper upon myocyte contraction. Tracking the triangle swimmer during contraction through subsequent video frames shows that (F) the relaxed construct contracts by (G) pulling the tail (tip) on the triangle in towards the base. (H) As the myocytes relax the triangle returns to its original shape producing a propulsive force that drives the construct forward. (I) The triangle's swimming velocity is a function of pacing rate. Spontaneous contractions produce 0.5 to 0.75 mm displacements spaced sporadically in time. Pacing at 0.5, 1.0 and 2.0 Hz produces cyclic contractions that reveal a maximum in swimming velocity of ~24 mm/min at 1.0 Hz pacing.

Measuring MTF Contractile Force

Rat adult skeletal (1 Hz pacing)
 850 μN peak contraction force
 43 $\mu\text{N}/\text{mm}^2$ (normalized to cross-sectional area)



Muscular Thin Film (1 Hz pacing)
 35 μN peak contraction force
 2800 $\mu\text{N}/\text{mm}^2$ (normalized to cross-sectional area)



- Continues to generate force at strains of 60% beyond the slack length
- PDMS component allows high strains without tissue damage
- Definitely combines attributes of muscle and polymer in a true composite structure